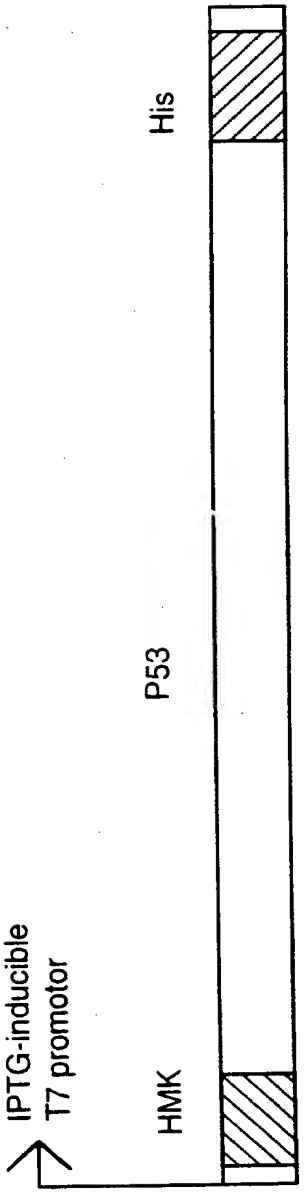

FIG. 1 Schematic representation of the bacterial p53-expression vector

Fig.2 Partial sequence of clone 1 and clone 2

A. Clone 1

GTATGAGGTGGAAGAAACAGAAGTGGTCATNAAGTCATACCAGAAGAACAGCGATCA
GGATGVNGHAGACAAAAAGAA------- ~400 bp----
GTATGAGGTGGAAGAAACAGAAGTGGTCATNAAGTCATACCAGAAGAACAGCGATCA
GGATGVNGHAGACAAAAAGAAAGCTTGGGNNCTATTCTATAGTGTCACCTAAAGACT
AGCTTG

B. Clone 2

CCTTCCGTTGAGGTATGTAGTACCCACTGATACCAGTATTGTAAATGACAGACACTGC
TTTCTCTGAGGGGAAGTCTGTTTCATTTTTGCACATTCCCGTTTGTAAA
----600 bp---CACCACCACACKCACARACACACKCCCAAAAAAAAACAAAAAACA
AACAAACAAAAAAAGCTTGGGCCT

A: Adenosine	R: A or G	K: G or T
C: Cytidine	Y: C or T	N: A, C, G or T
G: Guanosine	V: A, C or G	
T: Thymidine	H: A, C or T	

Fig.3 DNA sequence of the mouse mdmx cDNA

```
   1  GTGGCTCTTG CGAACTCTGG GTTTGAGAGG CCGGAACTGG TGCTGCCGTT
  51  GCTCGCAGTT TCAAAATGCA GTGCAGGCCT TAGGGTCTCC GGCTGCCACC
 101  CCTCCCCCAG CTAGGAGGGG GAGCGACTCA TGGAGCGGCC GTAAGTTTGC
 151  TAACTGTGGA GTCTTCACTG CCAAAATGAC ATCACATTCC ACCTCGGCCC
 201  AGTGTTCAGC ATCTGACAGT GCTTGCAGAA TTTCTTCGGA ACAAATTAGT
 251  CAGGTGCGGC CAAAACTGCA GCTTTTGAAG ATTTTGCATG CAGCAGGTGC
 301  GCAGGGGGAA GTATTCACCA TGAAAGAGGT AATGCACTAT CTAGGCCAGT
 351  ATATAATGGT GAAGCAGCTC TATGATCAAC AGGAGCAACA TATGGTATAC
 401  TGTGGTGGAG ATCTTTTGGG AGATCTACTT GGATGTCAGA GCTTTTCTGT
 451  GAAAGATCCA AGCCCTCTCT ATGACATGCT AAGAAAGAAT CTTGTTACAT
 501  CAGCTTCTAA TAACACAGAT GCTGCTCAGA CTCTCGCTCT CGCACAGGAT
 551  CACACTATGG ATTTTCCAAG TCAAGACCGA CTGAAGCACG GTGCAACAGA
 601  ATACTCCAAT CCCAGAAAAA GAACTGAAGA AGAGGATACT CACACACTGC
 651  CTACCTCACG ACATAAATGC AGAGACTCCA GAGCAGATGA AGACTTGATA
 701  GAACATTTAT CTCAAGATGA GACATCTAGG CTTGACCTTG ATTTTGAGGA
 751  GTGGGACGTT GCTGGCCTGC CTTGGTGGTT TCTAGGGAAT TTGAGAAACA
 801  ACTGTATTCC TAAAAGTAAT GGCTCAACTG ATTTACAGAC AAATCAGGAT
 851  ATAGGTACTG CCATTGTTTC AGACACTACG GATGATTTGT GGTTTTTAAA
 901  TGAGACCGTG TCAGAGCAAT TAGGTGTTGG AATAAAAGTT GAAGCTGCTA
 951  ATTCTGAGCA AACAAGTGAA GTAGGGAAAA CAAGTAACAA GAAGACGGTG
1001  GAGGTGGGAA AGGATGATGA TCTTGAGGAC TCCAGGTCCT TGagCGATGA
1051  TACTGACGTG GAACTTACCT CTGAGGATGA GTGGCAGTGT ACGGAATGCA
1101  AGAAGTTTAA TTCTCCAAGC AAGAGGTACT GTTTTCGTTG CTGGGCCTTG
1151  AGAAAGGATT GGTATTCGGA TTGTTCTAAA TTAACTCATT CCCTATCTAC
1201  ATCTAATATT ACTGCCATAC CTGAAAAGAA GGACAATGAA GGAATTGATG
1251  TTCCCGATTG TAGGAGAACC ATTTCAGCTC CTGTTGTTAG GCCTAAAGAT
1301  GGATATTTAA AGGAGGAAAA GCCCAGGTTT GACCCTTGCA ACTCAGTGGG
1351  ATTTTTGGAT TTGGCTCATA GTTCTGAAAG CCAGGAGATC ATCTCAAGCG
1401  CGAGAGAACA AACAGATATT TTTTCTGAGC AGAAAGCTGA AACAGAAAGT
1451  ATGGAAGATT TCCAGAATGT CTTGAAGCCG TGTAGCTTAT GTGAAAAAAG
1501  GCCTCGGGAT GGGAACATTA TTCATGGGAA GACGAGCCAT CTGACGACAT
1551  GTTTCCACTG TGCCAGGAGA CTGAAGAAGT CTGGGGCTTC GTGTCCTGTT
1601  TGTAAGAAAG AGATTCAGTT GGTTATTAAA GTTTTTATAG CATAGTTGAG
1651  TCAGTCACAG AGAAATACTA GGAGGACCAG GTCATTTATC AAAAAAAAAA
1701  A
```

Fig 4. Amino acid sequence of the putative mouse MDMX protein, and the alignment with the amino acid sequence of mouse MDM2 protein
| = identical amino acid
: = conserved amino acid
The p53-binding domain is depicted in Bold/Italic
The Zinc-finger motif around position 310-320 and the putative Ring finger around position 435-480 are indicated in Bold
The putative nucleotide binding site (451-453) is underlined

```
                10         20         30         40         50         59
Mdmx    MTSHSTSAQCSASDSACRI-SSEQISQVRPKLQLLKILHAAGAQGEVFTMKEVMHYLGQY
        |  :  :  |:   :::::|:::|  :||| : ||||  |||:|:::|||:::|||||:: |:|||
Mdm2    MCNTNMSVSTEGAASTSQIPASEQETLVRPKPLLLKLLKSVGAQNDTYTMKEIIFYIGQY
               10         20         30         40         50         60

       60         70         80         90         100        110        119
Mdmx    IMVKQLYDQQEQHMVYCGGDLLGDLLGCQSFSVKDPSPLYDMLRKNLV TSASNNTDAAQT
        ||:|:|||:::||:|||::|||||::| :|||||::: :|:|: :|||   | :: |:: :
Mdm2    IMTKRLYDEKQQHIVYCSNDLLGDVFGVPSFSVKEHRKIYAMIYRNLV --AVSQQDSGTS
               70         80         90         100        110

      120        130        140        150        160        170        179
Mdmx    LALAQDHTMDFPSQDRLKHGATEYSNPRKRTEEEDTHTLPTSRHKCRDSRADEDLIEHLS
        |: :: ::    : : || :: : :   |:::::  :  |:||::: |: :::|: :::|:
Mdm2    LSESRRQP---EGGSDLK-DPLQAPPEEKPSSSDLISRLSTSSRR-RSISETEENTDELP
       120          130        140        150        160        170

        180        190        200        210        220        230
Mdmx    --QDETSRLDLDFE-EWDVAGLPWWFLGNLRNNCIPKSNGSTDLQTNQDIGTAIVSDTTD
          :: ::| :|:|: : :: :|:    |:  ::: ::|::||: :::||:::::: ::::|
Mdm2    GERHRKRRRSLSFDPSLGLCELREMCSGG-TSSSSSSSSESTETPSHQDLDDGVSEHSGD
           180        190        200        210        220        230

         240        250        260        270        280        290
Mdmx    DLWFLNETVSEQLGVGIKVEAANSEQ--TSEVGKTSNKKTVEVGKDDDLEDSRSLSD--D
        |   :::||:|::|:::||: :||:   |: |:: :::: || : :  ::: | :|  :
Mdm2    CL--DQDSVSDQFSVEFEVESLDSEDYSLSDEGHELSDEDDEVYRVTVYQTGESDTDSFE
               240        250        260        270        280        290

             300        310        320        330        340        350
Mdmx    TDVELTSEDEWQCTECKKFNSPSKRYCFRCWALRKDWYSDCSKLTHSLSTSNITAIPEK-
        :| |:: :| |:|||:|:::|:|  ::| |||:||::| :| :| ::::::|: : : ::
Mdm2    GDPEISLADYWKCTSCNEMNPPLPSHCKRCWTLRENWLPD-DKGKDKVEISEKAKLENSA
               300        310        320        330        340

              360        370        380        390        400        410
Mdmx    KDNEGIDVPDCRRTISAPVVRPKDGYLKEEKPRFDPCNSVGFLDLAHSSESQEIISSARE
        :::||:|||| :: ::: :  | ::  :|||:  :|  | :  | :::|:| :|: |::|
Mdm2    QAEEGLDVPDGKKLTENDAKEPCAEEDSEEKAEQTP-LSQESDDYSQPSTSSSIVYSSQE
       350        360        370        380        390        400
```

```
            420         430         440         450         460
Mdmx   QTDIFSEQKAE-TESME-DFQ-NVLKPCSLCEKRPRDGNIIHGKTSHLTTCFHCARRLKK
       :: .:|:::: :||:| :|  |:::|| :|: ||:|  |:|||||:|| :|| ||:|||
Mdm2   SVKELKEETQHKDESVESSFSLNAIEPCVICQGRPKNGCIVHGKTGHLMSCFTCAKKLKK
      410         420         430         440         450         460

      470         480         490
Mdmx   SGASCPVCKKEIQLVIKVFIA
       :: :||||:: ||::: :::
Mdm2   RNKPCPVCRQPIQMIVLSYFN
      470         480
```

Fig. 5. Neucleotide sequence of the human MDMX cDNA isolated sofar.

AATTCGGCACGAGCTAGGATCTGTGACTGCCACCCCTCCCCCCACCCGGGCTCGGCGGGGGAGCG
ACTCATGGAGCTGCCGTAAGTTTTACCAACAGACTGCAGTTTCTTCACTACCAAAATGACATCA
TTTTCCACCTCTGCTCAGTGTTCAACATCTGACAGTGCTTGCAGGATCTCTCCTGGACAAATCAAT
CAGGTACGACCAAAACTGCCGCTTTTGAAGATTTTGCATGCAGCAGGTGCGCAAGGTGAAATGTT
CACTGTTAAAGAGGTCATGCACTAATTTAGGTCAGTACATAATGGTGAAGCAACTTTATGATCAG
C GGAGCAGCATATGGTATATTGTGGTGGAGATCTTTTGGGAGAACTACTGGGACGTCAGAGC
TTCTCCGTAAAGAACCCAAGCCCTCTCTATGATATGCTAAGAAAGAATCTTGTCACTTTAGCCACT
GCTACTACAGATGCTGCTCAGACTCTCGCTCTCGCACAGGATCACAGTATGGATATTCCAAGTC
AAGACCAACTGAAGCAAAGTGCAGAGGAAAGTTCCACTTCCAGAAAAAGAACTACAGAAGACGATA
TCCCCACACTGCCTACCTCAGAGCATAAATGCATACATTCTAGAGAAGATGAAGACTTAATTGAAAA
TTTAGCCCAAGATGAAACATCTAGGCTGGACCTTGGATTTGAGGAGTGGGATGTAGCTGGCCTGCC
TTGGTGGTTTTTAGGAAACTTGAGAAGCAACTATACACCTAGAAGTAATGGCTCAACTGATTTACA
GACAAATCAGGATGTGGGTACTGCCATTGTTTCAGATACTACAGATGACTTGTGGTTTTTGAAT
GAGTCAGTATCAGAGCAGTTAGGTGTTGGAATAAAAGTTGAAGCTGCTGATACTGAACAAACAAGT
GAAGAAGTAGGGAAAGTAAGTGACAAAAAGGTGATTGAAGTGGGAAAAAATGATGACCTGGAGG
ACTCTAAGTCCTTAAGTGATGATACCGATGTAGAGGTTACCTCTGAGGATGAGTGGCAGTGTAC
TGAATGCAAGAAATTTAACTCTCCAAGCAAGAGGTACTGTTTTCGTTGTTGGGCCTTGAGGAAGG
ATTGGTATTCAGATTGTTCAAAGTTAACCCATTCTCTCTCCACGTCTGATATCACTGCCATACCT
GAAAAGGAAAATGAAGGAAATGATGTCCCTGATTGTCGAAGAACCATTTCGGCTCCTGTCGTTAG
ACCTAAAGATGCGTATATAAAGAAAGAAAACTCCAAACTTTTTGATCCCTGCAACTCAGTGGAATT
CTTGGATTTGGCTCACAGTTCTGAAAGCCAAGAGACCATCTCAAGCATGGGAGAACAGTTAGATAA
CCTTTCTGAACAGAGAACAGATACAGAAAACATGGAGGATTGCCAGAATCTCTTGAAGCCATGTA
GCTTATGTGAGAAAAGACCACGAGACGGGAACATTATTCATGGAAGGACGGGCCATCTTGTCACTT
GTTTTCACTGTGCCAGAAGACTAAAGAAGGCTGGGGCTTCATGCCCTATTTGCAAGAAAGAGATT
CAGCTGGTTATTAAGGTTTTTATAGCATAATGGTAGTACGAACATAAAAATGCATTTATTCAGTT
CACTTACCACATTATTTGAAAATCAATCCTTTATTTAATTTTATTTCCAACCTGTCAGAGAATG
TTCTTAGGCATCAAAATCCAAGGTAGCTGTAAGAAAAATACTGGAGCTAACAATGAAGAACAGAAG
TAATCTGATTAGTCAAATTATTAAGTGCCATGGATTACTTTATGCAGCAGTCAGGTACATAGTT
AGGTGAACCAAAAGAAAAACTCTTGAAAACAAGAGATTTCTTCCATGCACATTTACAATATTGAGG
TATAATTAACATGATAAAGTGTTTCCTTCTAACGAGTTGTAGAAATCTGAGTAACCACCCAAAAAA
GCAATAGAATGTTTGTGTCACCCCAAAACACTCCCTTCTGCCCCTCTTCAGACAGTCCTTCAGCTA
TTTCATGGCTCTCACCCTAGTTTTTTTTTTTTTTTGCACTTTTTTTTTTCCGGGGGTATAGGGG
AGGTGTGGGGCGACAGGGTCTGTCTTGTTCTGTCTCCCAGGCTGAAGTGCAGTGAGTCAAGATT
GAGCCACTGCACTCCAGCCTGGGTGACAGCGCGAGACTCCATCTCAGAAAAAAAAAAAAAAAA
AAAAAAAACTAT

Fig. 6 Comparison of the amino acid sequences of human MDMX and mouse MDMX. Each protein consists of 489 amino acids.

```
hMDMX  MTSFSTSAQCSTSDSACRISPGQINQVRPKLPLLKILHAAGAQGEMFTVKEVMHYLGQYIMVK
mMDMX  MTSHSTSAQCSASDSACRISSEQISQVRPKLQLLKILHAAGAQGEVFTMKEVMHYLGQYIMVK

hMDMX  QLYDQQEQHMVYCGGDLLGELLGRQSFSVKNPSPLYDMLRKNLVTLATATPAQTLALAQDH
mMDMX  QLYDQQEQHMVYCGGDLLGDLLGCQSFSVKDPSPLYDMLRKNLVTSAGNNTDAAQTLALAQDH

hMDMX  SMDIPSQDLLKQSAEESSTSRKRTTEDDIPTTLPTSEHKCIHSREDEDLIENLAQDETSRLDLG
mMDMX  TMDFPSQDRLKHGATEYSNPRKRTEEEDTHTLPTSRHKCRDSRADEDLIEHLSQDETSRLDLD

hMDMX  FEEWDVAGLPWWFLGNLRSNYTPRSNGSTDLQTNQDVGTAIVSDTTDDLWFLNESVSEQLGVG
mMDMX  FEEWDVAGLPWWFLGNLRNNCIPKSNGSTDLQTNQDIGTAIVSDTTDDLWFLNETVSEQLGVG

hMDMX  IKVEAADTEQTSEEVGKVSDKKVIEVGKNDDLEDSKSLSDDTDVEVTSEDEWQCTECKKFNSP
mMDMX  IKVEAANSEQTSE.VGKTSNKKTVEVGKDDDLEDSRSLSDDTDVELTSEDEWQCTECKKFNSP

hMDMX  SKRYCFRCWALRKDWYSDCSKLTHSLSTSDITAIPE.KENEGNDVPDCRRTISAPVVRPKDAY
mMDMX  SKRYCFRCWALRKDWYSDCSKLTHSLSTSNITAIPEKKDNEGIDVPDCRRTISAPVVRPKDGY

hMDMX  IKKENSKLFNPCNSVEFLDLAHSSESQETISSMGEQLDNLSEQRTDTENMEDCQNLLKPCSLC
mMDMX  LKEEKPR.FDPCNSVGFLDLAHSSESQEIISSAREQTDIFSEQKAETESMEDFQNVLKPCSLC

hMDMX  EKRPRDGNIIHGRTGHLVTCFHCARRLKKAGASCPICKKEIQLVIKVFIA *
mMDMX  EKRPRDGNIIHGKTSHLTTCFHCARRLKKSGASCPVCKKEIQLVIKVFIA *
```

Fig. 7 Comparison of the amino acids sequences of human MDM2 and human MDMX.

```
hMDM2  MCNTNM.SVPTDGAVTTSQIP..AS.EQETLVRPKPLLLKLLKSVGAQKDTYT
hMDMX  .....MTSFSTSAQCSTSDSACRISPGQINQVRPKLPLLKILHAAGAQGEMFT

hMDM2  MKEVLFYLGQYIMTKRLYDEKQQHIVYCSNDLLGDLFGVPSFSVKEHRKIYTM
hMDMX  VKEVIHYLGQYIMVKQLYDQQEQHMVYCGGDLLGELLGCQSFSVKNPSPLYDM

hMDM2  IYRNLVVVNQQESSDSGTSVSENRCHLEGGSDQKDLVQELQEEKPSSSHL..
hMDMX  LRKNLVTLATATTDAAQTLALAQDHTMDIPS.QDQLKQSAEESSTSRKRTTE

hMDM2  ..VSRPSTSSRRRAISETEENSDELSGERQRKRHKSDSISLSFDE.......S
hMDMX  DDIPTLPTSEHKCIHSREDEDLIENLAQDETSR.....LDLGFEEWDVAGLPW

hMDM2  LALCVIREICCERSSSSESTGTPSNPDLDAGVSEHSGD..WLDQDSVSDQFSV
hMDMX  WFLGNLRSNYTPRSNG..STDLQTNQDVGTAIVSDTTDDLWFLNESVSEQLGV

hMDM2  EFEVESLDSEDYSLSEEGQELSDEDDEVYQVTVYQA.GESDTDSFEEDPEISL
hMDMX  GIKVEAADTEQ..TSEEVGKVS..DKKVIEVGKNDDLEDSKSLSDDTDVEVTS

hMDM2  ADYWKCTSCNEMNPPLPSHCNRCWALRENWLPEDKGKDKGEISEKAKLENSTQ
hMDMX  EDEWQCTECKKFNSPSKRYCFRCWALRKDWYS.DCSKLTHSLSTSDITAIPEK

hMDM2  AEEGFDVPDCKKTI...VNDSRESCVEENDDKITQASQSQESEDYSQPSTSSS
hMDMX  ENEGNDVPDCRRTISAPVVRPKDAYIKKENSKLFNPCNSVEFLDLAHSSESQE

hMDM2  IIYSSQEDVKEF..EREETQDKEESVESSLPLNAIEPCVICQGRPKNGCIVHG
hMDMX  TISSMGEQLDNLSEQRTDTENMEDC......QNLLKPCSLCEKRPRDGNIIHG

hMDM2  KTGHLMACFTCAKKLKKRNKPCPVCRQPIQMIVLTYFP *
hMDMX  RTGHLVTCFHCARRLKKAGASCPICKKEIQLVIKVFIA *
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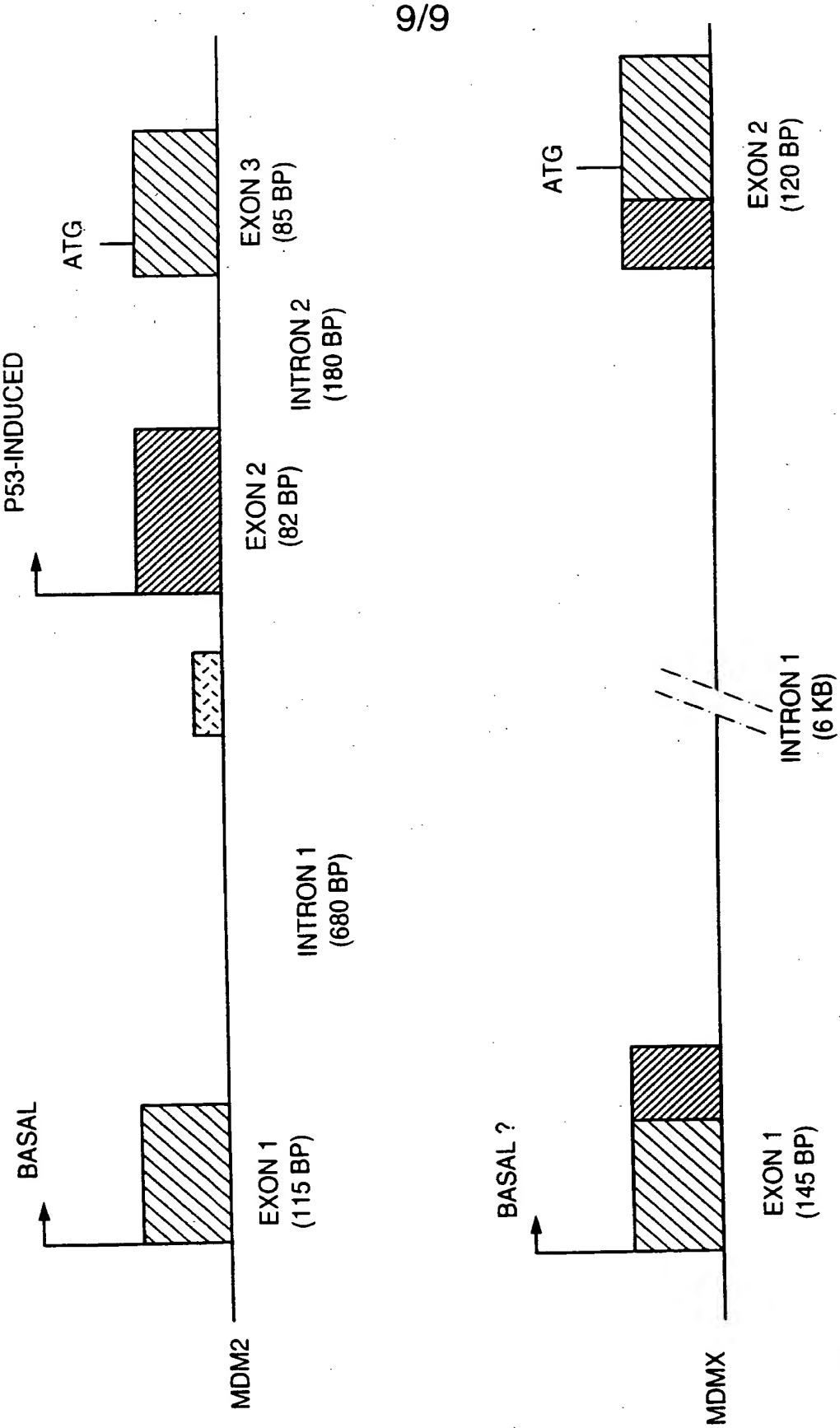
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EXON 3
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MDMX
BASAL ?
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INTRON 1
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ATG
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FIG. 8